Supplementary Material

Glyphosate exposure and urinary oxidative stress biomarkers in the Agricultural Health Study

Table of Contents

Supplementary Methods **2**
Selection and recruitment of nonfarmers in the BEEA study 2
Questionnaire-based glyphosate exposure assessment 2
Glyphosate use-defined study groups 3
Urinary glyphosate measurements 4
Urinary oxidative stress biomarker measurements 5
8-hydroxy-2'-deoxyguanosine (8-OHdG) 5
Free 8-iso-prostaglandin-F2α (8-Isoprostane) 6
Malondialdehyde (MDA) 7
Supplementary References **8**
Supplementary Tables **9**
Supplementary Table 1 9
Supplementary Table 2 10
Supplementary Table 3 11
Supplementary Table 4 12
Supplementary Table 5 13
Supplementary Table 6 14
Supplementary Table 7 15
Supplementary Table 8 17

Supplementary Methods

Selection and recruitment of nonfarmers in the BEEA study

In addition to the 1681 male farmers, the Biomarkers of Exposure and Effect in Agriculture (BEEA) study enrolled 211 male nonfarmers who were identified using voter registration lists and recruited from ten counties located outside major metropolitan areas in each state (Iowa or North Carolina) from which most BEEA farmers were recruited. The BEEA nonfarmers were selected to have similar distributions as the BEEA farmers in terms of age, race and ethnicity (Black, White, or other [American Indian or Alaska Native, Asian, and Native Hawaiian or other Pacific Islander]), and geographic location (state and county) of residence. To be eligible for inclusion, the nonfarmers had to be ≥50 years of age at the time of enrollment, have no history of cancer (except non-melanoma skin cancer), have no blood clotting disorder such as hemophilia, and have not lived or worked on a farm or held a job that involved mixing, loading, or applying pesticides within the last 10 years or for longer than 12 months since the age of 18. In addition, nonfarmers who worked in any of the following occupations or industries in the last 12 months were considered ineligible for participation in BEEA due to concerns about potential occupational exposures to carcinogens: construction, meat packing, janitorial services, furniture making, mechanic or gas station attendant, landscaping, painting, or welding.

Questionnaire-based glyphosate exposure assessment

Information on occupational glyphosate use among farmers was obtained from questionnaires administered at Agricultural Health Study (AHS) enrollment (1993-1997) and 2 follow-up interviews (phase 2 [1999-2003] and phase 3 [2005-2010]), as well as at the BEEA enrollment interview (2010-2018). At AHS enrollment, participants were asked to report whether they have ever personally mixed or applied specific pesticides, including glyphosate, during their lifetime, and if so, the number of days in an average year and the number of years each pesticide was used. In each of the follow-up interviews, participants provided updated information on pesticide use since enrollment or the previous interview. At BEEA enrollment, participating farmers reported detailed information on occupational use of specific pesticides during the 12-month period preceding the interview, including total number of days of use in the last 12 months and dates of the most recent uses of each pesticide. The BEEA questionnaire also collected information about home and garden use of pesticides in the last 12 months from both farmers and nonfarmers.

Combining and summing data across all 4 questionnaires, we calculated cumulative total lifetime days of occupational glyphosate use by multiplying the number of days of use per year by the number of years used. Intensity-weighted lifetime days of use was then calculated by multiplying total lifetime days of use by an exposure intensity score that accounts for factors (reported by participants in the questionnaires) known to influence pesticide exposure, such as mixing and loading of pesticides, application method, equipment repair, and personal protective equipment use (1). Similarly, based on responses from the BEEA questionnaire, we also calculated intensity-weighted days of occupational glyphosate use in the last 12 months by multiplying the number of days of use in the last 12 months by the exposure intensity score.

Glyphosate use-defined study groups

For this investigation, we selected 4 subgroups of BEEA participants based on their reported glyphosate use. First, as a "recently exposed" group, we selected 100 farmers whose most recent date of occupational glyphosate use was within the 7-day period prior to the BEEA enrollment interview, regardless of their lifetime occupational glyphosate use. We later excluded 2 of the 100 selected farmers from the analyses because their most recent glyphosate use was on the same day as the interview, and, presumably after, the time of urine collection (first-morning-void samples), and their next most recent date of glyphosate use was outside the 7-day period. Second, we randomly selected a “high lifetime-exposed” group of 70 farmers who were in the top 80th percentile of both total lifetime days (≥197) and intensity-weighted lifetime days (≥11,334.29) of occupational glyphosate use among all BEEA farmers but reported no occupational glyphosate use in the 7 days preceding the interview. Third, as a minimally exposed comparison group of farmers (“farming controls”), we selected 100 farmers with no or very little lifetime occupational glyphosate use, defined as no use at BEEA enrollment or the AHS phase 3 interview (ie, no use since after the phase 2 interview in 1999-2003) and in the lowest tertiles of both total lifetime days (<14.5) and intensity-weighted lifetime days (<677.25) of occupational glyphosate use among all BEEA farmers (note: 47 of the 100 farmers selected for this group had never used glyphosate occupationally during their lifetime). Finally, as an external comparison group, we selected 100 “nonfarming controls” (described above in “Selection and recruitment of nonfarmers in the BEEA study”) who reported no home or garden use of glyphosate during the 7 days before the BEEA interview.

Urinary glyphosate measurements

Urinary glyphosate concentrations were measured at the Centers for Disease Control and Prevention (CDC; Atlanta, GA) using an ion chromatography isotope dilution-tandem mass spectrometry method as described previously (2) and briefly below. First, to prepare calibration standards, a glyphosate solution (Supelco Inc., Bellefonte, PA) was serially diluted with in-house ultrapure water (AQUA Solutions, Lab Water Systems, Jasper, GA) to concentrations ranging from 0.1 to 60 µg/L (note: the 0.1 µg/L calibrator was used as an “instrument ready check” and excluded from the calibration curve, which was in the range of 0.2-60 µg/L). Next, test samples were prepared by adding 50 µL of an internal standard of isotope-labeled (^{15}N, 2–^{13}C) glyphosate (Cambridge Isotope Laboratories, Andover, MA) and 200 µL of water to 200 µL of the urine sample or the calibration standard. Ion chromatography was then performed using a Dionex ICS-5000+ system (Thermo Fisher Scientific, Sunnyvale, CA) with a dual-column and switching valve assembly for analyte extraction and chromatographic separation, followed by tandem mass spectrometry analysis using an AB Sciex 5500 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a TurboIonSpray source. The CDC laboratory is certified by the Health Care Financing Administration to comply with the requirements set forth in the Clinical Laboratory Improvement Amendments (CLIA) of 1988 and is recertified every 2 years. At CDC, analytical measurements were conducted following strict quality control/quality assurance CLIA guidelines, and included successful participation in external quality assessment schemes (eg, G-EQUAS, OSEQAS) since 2019 to continuously demonstrate the accuracy and precision of the analytical methods. Furthermore, along with the study samples, each analytical run included high- and low-concentration quality control materials (QCs) and reagent blanks to assure the accuracy and reliability of the data. The concentrations of the QCs were evaluated using standard statistical probability rules (3). If the QC samples failed the statistical evaluation, all of the samples in the run were re-extracted.

Urinary oxidative stress biomarker measurements

8-hydroxy-2'-deoxyguanosine (8-OHdG)

Urinary 8-OHdG concentrations were quantified using the DNA/RNA Oxidative Damage (Clone 7E6.9) enzyme-linked immunosorbent assay (ELISA) Kit (Item No. 501130; Cayman Chemical, Ann Arbor, MI), a competitive ELISA based on the competition between oxidatively damaged guanine species and an 8-hydroxyguanosine acetylcholinesterase (AChE) conjugate for a limited amount of monoclonal antibody. To prepare the standard curve, a standard solution of 8-OHdG in ethanol was serially diluted with ELISA buffer to concentrations ranging from 0.156 to 20 ng/mL. Urine samples were also diluted as appropriate (at least 2 dilutions for each sample, at 1:10 and 1:40) in ELISA buffer so that the absorbances would fall within the linear range of the standard curve. To perform the assay, 50 μL of each standard was added in duplicate and 50 μL of each diluted urine sample was added in triplicate to wells of a goat anti-mouse IgG coated 96-well plate. Each plate also contained 2 blank, 2 non-specific binding (NSB), and 3 maximum binding (B_0) wells, with 100 and 50 μL of ELISA buffer added to each of the NSB and B_0 wells, respectively. Subsequently, 50 μL of the DNA/RNA Oxidative Damage (Clone 7E6.9) ELISA AChE tracer was added to each well, except the blank wells, and 50 μL of the DNA/RNA Oxidative Damage (Clone 7E6.9) ELISA monoclonal antibody was added to each well, except the blank and NSB wells. All plates were covered with plastic film and incubated overnight at 4 °C. Following incubation, plates were rinsed several times to remove unbound material, and 200 μL of Ellman's reagent (containing the substrate to AChE) was added to each well. Plates were then incubated on an orbital shaker for 90 min and read using a Spectramax 340PC384 Microplate Reader (Molecular Devices, San Jose, CA) at a wavelength of 414 nm. The concentration in each sample was determined using a 4-parameter logistic fit of the standard concentrations vs %Bound/Maximum Bound (%B/B_0; ratio of the absorbance of a sample or standard well to that of the B_0 well). All concentrations fell well above the assay sensitivity (defined as 80% B/B_0) of 0.45 ng/mL.

Free 8-iso-prostaglandin-F2α (8-Isoprostane)

Urinary free 8-isoprostane concentrations were quantified using the 8-Isoprostane ELISA Kit (Item No. 516351; Cayman Chemical), a competitive ELISA based on the competition between 8-isoprostane and an 8-isoprostane-AChE conjugate for a limited number of 8-isoprostane-specific rabbit antiserum binding sites. Prior to assay, samples were first purified using a C-18 solid phase extraction purification method, and samples were eluted with ethyl acetate containing 1% methanol. Eluted samples were then dried under a gentle stream of nitrogen to evaporate the ethyl acetate solution, resuspended in ELISA buffer, and diluted as necessary (at least 2 dilutions for each sample, at 1:5 and 1:20). To prepare the standard curve, the 8-isoprostane ELISA standard was serially diluted with ELISA buffer to concentrations ranging from 0.8 to 500 pg/mL. To perform the assay, 50 μL of each standard was added in duplicate and 50 μL of each diluted sample was added in triplicate to wells of a mouse anti-rabbit IgG coated 96-well plate. Each plate also contained 2 blank, 2 NSB, and 3 B_0 wells, with 100 and 50 μL of ELISA buffer added to each of the NSB and B_0 wells, respectively. Subsequently, 50 μL of the 8-isoprostane AChE tracer was added to each well, except the blank wells, and 50 μL of the 8-isoprostane antiserum was added to each well, except the blank and NSB wells. All plates were covered with plastic film and incubated overnight at 4 °C. Following incubation, plates were rinsed several times to remove unbound material, and 200 μL of Ellman's reagent was added to each well. Plates were then incubated on an orbital shaker for 90 min and read using a Spectramax 340PC384 Microplate Reader (Molecular Devices) at a wavelength of 414 nm. The concentration in each sample was determined using a 4-parameter logistic fit of the standard concentrations vs $\%B/B_0$. All concentrations fell well above the assay sensitivity of 3 pg/mL.

Malondialdehyde (MDA)

Urinary MDA concentrations were quantified using the TBARS (TCA Method) Assay Kit (Item No. 700870; Cayman Chemical), which was based on the reaction with thiobarbituric acid (TBA) to form MDA-TBA adducts that can be measured fluorometrically. First, the fluorometric standards were prepared by diluting the MDA standard with water to concentrations of 0, 0.0625, 0.125, 0.25, 0.5, 1, 2.5, and 5 μM. To perform the assay, 100 μL of each standard or urine sample (undiluted) was added to a microcentrifuge vial, followed by additions of 100 μL of 10% trichloroacetic acid and 800 μL of a color reagent to each vial. After mixing using a vortex, capped vials were placed in foam and boiled for 1 h in vigorously boiling water, followed by incubation on ice for 10 min to stop the reaction. The vials were then centrifuged for 10 min at 1,600 × g at 4 °C. Subsequently, 200 μL (in duplicate for standards and triplicate for samples) were removed from each vial without disturbing the pellet and transferred to a black 96-well solid plate. Fluorescence was read using the Tecan Safire2 microplate reader (Männedorf, Switzerland) at an excitation wavelength of 530 nm and an emission wavelength of 550 nm, with the sensitivity set to high and the excitation and emission bandwidths set to 10 nm. The concentration in each sample was calculated from the standard curve using the following formula: MDA (μM) = [(corrected fluorescence – y-intercept) ÷ slope], where the corrected fluorescence was determined by subtracting the background fluorescence (ie, fluorescence value of the 0 μM standard) from the measured fluorescence of each standard or sample.

Supplementary References

1. Coble J, Thomas KW, Hines CJ, et al. An updated algorithm for estimation of pesticide exposure intensity in the Agricultural Health Study. *Int J Environ Res Public Health*. 2011;8(12):4608–4622.

2. Schütze A, Morales-Agudelo P, Vidal M, et al. Quantification of glyphosate and other organophosphorus compounds in human urine via ion chromatography isotope dilution tandem mass spectrometry. *Chemosphere*. 2021;274:129427.

3. Caudill SP, Schleicher RL, Pirkle JL. Multi-rule quality control for the age-related eye disease study. *Stat Med*. 2008;27(20):4094–4106.

Supplementary Tables

Supplementary Table 1. Selected characteristics of participants according to quartiles of urinary glyphosate concentrations in the BEEA study

Characteristics[a]	Quartile 1 (n = 93)	Quartile 2 (n = 91)	Quartile 3 (n = 92)	Quartile 4 (n = 92)
Age, mean (SD), y	63.0 (9.9)	63.9 (8.8)	63.3 (9.1)	63.9 (8.6)
BMI, mean (SD), kg/m^2	30.1 (6.4)	30.5 (6.2)	29.1 (5.1)	28.5 (4.0)
State				
Iowa	69 (74.2)	70 (76.9)	68 (73.9)	71 (77.2)
North Carolina	24 (25.8)	21 (23.1)	24 (26.1)	21 (22.8)
Race				
Black	1 (1.1)	0 (0.0)	1 (1.1)	1 (1.1)
White	91 (97.8)	91 (100.0)	89 (96.7)	90 (97.8)
Other[b]	1 (1.1)	0 (0.0)	2 (2.2)	1 (1.1)
Season of urine collection				
April-September	57 (61.3)	63 (69.2)	62 (67.4)	73 (79.3)
October-March (off-season)	36 (38.7)	28 (30.8)	30 (32.6)	19 (20.7)
Time of urine collection				
Before 4:00 AM	21 (22.6)	13 (14.3)	14 (15.2)	12 (13.0)
4:00-5:59 AM	42 (45.2)	34 (37.4)	32 (34.8)	32 (34.8)
6:00 AM or later	30 (32.3)	44 (48.4)	46 (50.0)	48 (52.2)
Smoking status				
Never	54 (58.1)	50 (54.9)	56 (60.9)	55 (59.8)
Former	36 (38.7)	31 (34.1)	34 (37.0)	35 (38.0)
Current	3 (3.2)	10 (11.0)	2 (2.2)	2 (2.2)
Alcohol consumption (last 7 d)[c]				
None	48 (51.6)	41 (45.1)	39 (42.4)	45 (48.9)
1-6 servings	22 (23.7)	30 (33.0)	34 (37.0)	33 (35.9)
≥7 servings	23 (24.7)	20 (22.0)	19 (20.7)	14 (15.2)
Recent NSAID use (last 7 d)[d]				
No	35 (37.6)	26 (28.6)	32 (34.8)	40 (43.5)
Yes	58 (62.4)	65 (71.4)	60 (65.2)	52 (56.5)
Recent infection (last 7 d)[e]				
No	84 (90.3)	81 (89.0)	83 (90.2)	78 (84.8)
Yes	9 (9.7)	10 (11.0)	9 (9.8)	14 (15.2)
History of diabetes				
No	81 (87.1)	76 (83.5)	77 (83.7)	83 (90.2)
Yes	12 (12.9)	15 (16.5)	15 (16.3)	9 (9.8)
History of hypertension and/or heart disease				
No	43 (46.2)	34 (37.4)	40 (43.5)	58 (63.0)
Yes	50 (53.8)	57 (62.6)	52 (56.5)	34 (37.0)
Home and/or garden glyphosate use				
Did not use in the last 12 mo	55 (59.1)	49 (53.8)	54 (58.7)	59 (64.1)
Used in the last 12 mo	38 (40.9)	42 (46.2)	38 (41.3)	33 (35.9)
Occupational 2,4-D use				
Did not use in the last 12 mo	66 (71.0)	66 (72.5)	59 (64.1)	40 (43.5)
8-365 days ago	24 (25.8)	18 (19.8)	23 (25.0)	42 (45.7)
≤7 days ago	3 (3.2)	7 (7.7)	10 (10.9)	10 (10.9)

[a] Presented as frequencies and percentages (%) unless otherwise specified. 2,4-D = 2,4-dichlorophenoxyacetic acid; BEEA = Biomarkers of Exposure and Effect in Agriculture; BMI = body mass index; NSAID = nonsteroidal anti-inflammatory drug.

[b] American Indian or Alaska Native (n = 1) or not reported (n = 3).

[c] Number of servings of alcoholic beverages in the last 7 days. One serving of an alcoholic beverage was defined as 12 fluid ounces of beer, 5 fluid ounces of wine, or 1.5 fluid ounces of hard liquor.

[d] Use of any aspirin- or ibuprofen-containing products in the last 7 days.

[e] Having a cold, flu, or other infection during the last 7 days.

Supplementary Table 2. Urinary concentrations (unadjusted and creatinine-corrected) of glyphosate and oxidative stress biomarkers among glyphosate-exposed and control groups in the BEEA study

Urinary marker	Recently exposed (n = 98)	High lifetime-exposed (n = 70)	Farming controls (n = 100)	Nonfarming controls (n = 100)
Glyphosate				
No. (%) ≥LOD[a]	89 (90.8%)	65 (92.9%)	88 (88.0%)	81 (81.0%)
Unadjusted, µg/L				
Median (IQR)	0.77 (0.40–1.44)	0.55 (0.29–1.12)	0.49 (0.27–0.76)	0.38 (0.24–0.62)
Geometric mean (95% CI)	0.89 (0.70 to 1.13)	0.59 (0.48 to 0.73)	0.46 (0.40 to 0.52)	0.39 (0.34 to 0.46)
Creatinine-corrected, µg/g creatinine				
Median (IQR)	0.72 (0.39–1.52)	0.47 (0.30–0.79)	0.41 (0.28–0.65)	0.37 (0.24–0.55)
Geometric mean (95% CI)	0.79 (0.64 to 0.98)	0.51 (0.43 to 0.60)	0.42 (0.37 to 0.47)	0.37 (0.33 to 0.42)
8-OHdG				
Unadjusted, µg/L				
Median (IQR)	11.58 (8.12–14.68)	12.52 (8.73–16.02)	11.34 (7.84–15.87)	10.44 (6.57–14.60)
Geometric mean (95% CI)	10.72 (9.70 to 11.83)	11.58 (10.35 to 12.96)	10.90 (9.77 to 12.16)	10.19 (9.17 to 11.33)
Creatinine-corrected, µg/g creatinine				
Median (IQR)	9.73 (7.46–12.47)	10.03 (8.05–12.31)	10.13 (7.64–12.92)	9.39 (7.71–11.88)
Geometric mean (95% CI)	9.60 (9.01 to 10.23)	9.93 (9.21 to 10.71)	10.03 (9.37 to 10.73)	9.66 (9.01 to 10.35)
8-isoprostane				
Unadjusted, µg/L				
Median (IQR)	0.58 (0.38–0.88)	0.60 (0.36–0.85)	0.53 (0.37–0.88)	0.56 (0.30–0.85)
Geometric mean (95% CI)	0.57 (0.50 to 0.64)	0.58 (0.49 to 0.69)	0.55 (0.47 to 0.64)	0.53 (0.46 to 0.62)
Creatinine-corrected, µg/g creatinine				
Median (IQR)	0.49 (0.40–0.61)	0.48 (0.38–0.63)	0.49 (0.38–0.68)	0.49 (0.39–0.65)
Geometric mean (95% CI)	0.51 (0.47 to 0.55)	0.50 (0.45 to 0.55)	0.51 (0.46 to 0.56)	0.50 (0.46 to 0.55)
MDA				
Unadjusted, µM				
Median (IQR)	1.68 (1.21–2.25)	1.70 (1.26–2.50)	1.67 (1.09–2.54)	1.52 (1.13–2.33)
Geometric mean (95% CI)	1.69 (1.52 to 1.88)	1.69 (1.49 to 1.91)	1.66 (1.47 to 1.88)	1.57 (1.38 to 1.78)
Creatinine-corrected, µmol/g creatinine				
Median (IQR)	1.51 (1.08–1.84)	1.37 (1.08–1.84)	1.45 (1.05–2.06)	1.40 (1.07–1.87)
Geometric mean (95% CI)	1.51 (1.39 to 1.65)	1.45 (1.30 to 1.61)	1.53 (1.38 to 1.69)	1.48 (1.35 to 1.63)

[a] Number (percentage) of participants with urinary glyphosate concentrations above the limit of detection (ie, ≥0.2 µg/L). 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; BEEA = Biomarkers of Exposure and Effect in Agriculture; CI = confidence interval; IQR = interquartile range; LOD = limit of detection; MDA = malondialdehyde.

Supplementary Table 3. Spearman correlation coefficients (ρ) for urinary concentrations (unadjusted or creatinine-corrected) of glyphosate and oxidative stress biomarkers, overall and by study group in the BEEA study[a]

	ρ for unadjusted concentrations				ρ for creatinine-corrected concentrations			
Urinary marker	Glyphosate	8-OHdG	8-isoprostane	MDA	Glyphosate	8-OHdG	8-isoprostane	MDA
Overall (n = 368)								
Glyphosate	1.00				1.00			
8-OHdG	0.51	1.00			0.16	1.00		
8-isoprostane	0.43	0.69	1.00		–0.02	0.10	1.00	
MDA	0.47	0.64	0.62	1.00	0.18	0.31	0.14	1.00
Recently exposed (n = 98)								
Glyphosate	1.00				1.00			
8-OHdG	0.45	1.00			0.14	1.00		
8-isoprostane	0.26	0.60	1.00		–0.17	<0.01	1.00	
MDA	0.43	0.64	0.61	1.00	0.09	0.35	0.12	1.00
High lifetime-exposed (n = 70)								
Glyphosate	1.00				1.00			
8-OHdG	0.50	1.00			–0.12	1.00		
8-isoprostane	0.52	0.75	1.00		–0.06	0.21	1.00	
MDA	0.56	0.60	0.62	1.00	0.11	0.23	0.06	1.00
Farming controls (n = 100)								
Glyphosate	1.00				1.00			
8-OHdG	0.52	1.00			0.17	1.00		
8-isoprostane	0.46	0.69	1.00		0.02	0.19	1.00	
MDA	0.46	0.69	0.61	1.00	0.22	0.37	0.18	1.00
Nonfarming controls (n = 100)								
Glyphosate	1.00				1.00			
8-OHdG	0.64	1.00			0.32	1.00		
8-isoprostane	0.53	0.73	1.00		0.07	0.02	1.00	
MDA	0.54	0.63	0.66	1.00	0.21	0.25	0.18	1.00

[a] 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; BEEA = Biomarkers of Exposure and Effect in Agriculture; MDA = malondialdehyde.

Supplementary Table 4. Associations between urinary glyphosate and oxidative stress biomarker concentrations in the BEEA study, by study group

Urinary glyphosate concentration (μg/L)[a]	No.	Fully adjusted GMR (95% CI)[b] 8-OHdG	8-isoprostane	MDA
Recently exposed				
Quartile 1 (<LOD-0.289)	12	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	18	0.98 (0.76 to 1.26)	1.00 (0.75 to 1.33)	1.09 (0.77 to 1.55)
Quartile 3 (0.507-0.933)	25	1.18 (0.94 to 1.49)	1.06 (0.81 to 1.38)	1.18 (0.86 to 1.63)
Quartile 4 (0.934-35.2)	43	1.23 (0.97 to 1.57)	0.96 (0.73 to 1.27)	1.19 (0.85 to 1.66)
P_{trend}[c]		.03	.53	.43
Continuous[d]	98	1.02 (0.97 to 1.06)	0.97 (0.92 to 1.02)	0.99 (0.94 to 1.06)
High lifetime-exposed				
Quartile 1 (<LOD-0.289)	18	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	16	0.95 (0.76 to 1.19)	0.94 (0.69 to 1.28)	1.22 (0.90 to 1.65)
Quartile 3 (0.507-0.933)	14	0.97 (0.75 to 1.24)	1.07 (0.75 to 1.52)	1.41 (1.00 to 1.98)
Quartile 4 (0.934-35.2)	22	0.97 (0.75 to 1.24)	0.99 (0.70 to 1.40)	1.18 (0.84 to 1.65)
P_{trend}[c]		.92	.97	.73
Continuous[d]	70	0.99 (0.91 to 1.07)	1.00 (0.89 to 1.11)	1.04 (0.93 to 1.16)
Farming controls				
Quartile 1 (<LOD-0.289)	28	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	24	1.08 (0.87 to 1.34)	0.89 (0.63 to 1.26)	1.23 (0.92 to 1.66)
Quartile 3 (0.507-0.933)	34	1.10 (0.88 to 1.38)	0.90 (0.63 to 1.28)	1.17 (0.86 to 1.60)
Quartile 4 (0.934-35.2)	14	1.08 (0.81 to 1.42)	0.78 (0.50 to 1.22)	1.08 (0.73 to 1.59)
P_{trend}[c]		.74	.32	.93
Continuous[d]	100	1.03 (0.94 to 1.13)	0.95 (0.82 to 1.10)	1.02 (0.90 to 1.17)
Nonfarming controls				
Quartile 1 (<LOD-0.289)	35	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	33	1.09 (0.91 to 1.31)	1.04 (0.83 to 1.30)	1.06 (0.82 to 1.36)
Quartile 3 (0.507-0.933)	19	1.47 (1.16 to 1.87)	1.35 (1.00 to 1.81)	1.23 (0.87 to 1.72)
Quartile 4 (0.934-35.2)	13	1.29 (1.00 to 1.67)	1.12 (0.81 to 1.54)	1.17 (0.81 to 1.68)
P_{trend}[c]		.06	.48	.39
Continuous[d]	100	1.09 (1.01 to 1.18)	1.05 (0.96 to 1.16)	1.07 (0.97 to 1.19)

[a] Quartiles are based on the distribution of urinary glyphosate concentrations among all participants (n = 368) for comparison with results presented in Table 2. 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; BEEA = Biomarkers of Exposure and Effect in Agriculture; CI = confidence interval; GMR = geometric mean ratio; LOD = limit of detection; MDA = malondialdehyde.

[b] Adjusted for age (continuous; years), natural log-transformed urinary creatinine concentration (continuous; mg/dL), state (Iowa, North Carolina), season of urine collection (April-September, October-March), time of urine collection (before 4:00 AM, 4:00-5:59 AM, 6:00 AM or later), body mass index (continuous; kg/m^2), smoking status (never, former, current), alcohol consumption (0, 1-6, ≥7 servings in the last 7 days), nonsteroidal anti-inflammatory drug use in the last 7 days (no, yes), infection in the last 7 days (no, yes), history of diabetes (no, yes), history of hypertension and/or heart disease (no, yes), and occupational 2,4-dichlorophenoxyacetic acid use (did not use in the last 12 months, 8-365 days ago, ≤7 days ago).

[c] Calculated by modeling within-quartile median values as a continuous variable.

[d] Per 1-unit increase in log_2-transformed urinary glyphosate concentration, corresponding to a doubling in urinary glyphosate concentration.

Supplementary Table 5. Age and creatinine adjusted associations between recent occupational glyphosate use (last 7 days) and urinary oxidative stress biomarker concentrations in the BEEA study

			Age and creatinine adjusted GMR (95% CI)[a]		
Glyphosate use	No.	Geometric mean concentration (95% CI)	Compared with nonfarming controls	Compared with farming controls	Among recently exposed only
8-OHdG (µg/L)					
Nonfarming controls	100	10.2 (9.2 to 11.3)	1.00 (Referent)	—	—
Farming controls	100	10.9 (9.8 to 12.2)	—	1.00 (Referent)	—
Recently exposed	98	10.7 (9.7 to 11.8)	1.01 (0.93 to 1.10)	0.97 (0.89 to 1.05)	—
Days since last use					
5-7	42	9.4 (7.8 to 11.2)	0.95 (0.86 to 1.06)	0.92 (0.82 to 1.03)	1.00 (Referent)
2-4	35	11.0 (9.5 to 12.7)	1.03 (0.91 to 1.15)	0.98 (0.87 to 1.10)	1.08 (0.95 to 1.23)
≤1	21	13.5 (11.8 to 15.4)	1.10 (0.96 to 1.27)	1.05 (0.91 to 1.21)	1.16 (1.00 to 1.36)
8-isoprostane (µg/L)					
Nonfarming controls	100	0.53 (0.46 to 0.62)	1.00 (Referent)	—	—
Farming controls	100	0.55 (0.47 to 0.64)	—	1.00 (Referent)	—
Recently exposed	98	0.57 (0.50 to 0.64)	1.01 (0.91 to 1.12)	1.00 (0.88 to 1.14)	—
Days since last use					
5-7	42	0.53 (0.43 to 0.64)	1.06 (0.92 to 1.21)	1.04 (0.89 to 1.23)	1.00 (Referent)
2-4	35	0.57 (0.46 to 0.70)	0.99 (0.85 to 1.15)	0.98 (0.83 to 1.17)	0.94 (0.80 to 1.10)
≤1	21	0.65 (0.50 to 0.85)	0.95 (0.79 to 1.14)	0.95 (0.77 to 1.18)	0.91 (0.75 to 1.10)
MDA (µM)					
Nonfarming controls	100	1.57 (1.38 to 1.78)	1.00 (Referent)	—	—
Farming controls	100	1.66 (1.47 to 1.88)	—	1.00 (Referent)	—
Recently exposed	98	1.69 (1.52 to 1.88)	1.04 (0.92 to 1.16)	1.01 (0.89 to 1.14)	—
Days since last use					
5-7	42	1.51 (1.26 to 1.80)	1.00 (0.86 to 1.16)	0.97 (0.83 to 1.13)	1.00 (Referent)
2-4	35	1.61 (1.37 to 1.90)	0.99 (0.84 to 1.16)	0.96 (0.81 to 1.13)	0.99 (0.83 to 1.18)
≤1	21	2.29 (1.83 to 2.88)	1.22 (1.01 to 1.49)	1.19 (0.97 to 1.46)	1.24 (1.01 to 1.52)

[a] Adjusted for age (continuous; years) and natural log-transformed urinary creatinine concentration (continuous; mg/dL). 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; BEEA = Biomarkers of Exposure and Effect in Agriculture; CI = confidence interval; GMR = geometric mean ratio; MDA = malondialdehyde.

Supplementary Table 6. Age and creatinine adjusted associations of occupational glyphosate use in the last 12 months and cumulative lifetime occupational glyphosate use with urinary oxidative stress biomarker concentrations in the BEEA study

Glyphosate use	No.	Geometric mean concentration (95% CI)	Age and creatinine adjusted GMR (95% CI)[a]		
			Compared with nonfarming controls	Compared with farming controls	Glyphosate-exposed farmers only
8-OHdG (μg/L)					
Nonfarming controls	100	10.2 (9.2 to 11.3)	1.00 (Referent)	—	—
Farming controls	100	10.9 (9.8 to 12.2)	—	1.00 (Referent)	—
Last 12-month use[b]					
Tertile 1	56	11.0 (9.5 to 12.6)	1.05 (0.96 to 1.16)	1.01 (0.92 to 1.11)	1.00 (Referent)
Tertile 2	56	11.0 (9.7 to 12.5)	0.97 (0.88 to 1.07)	0.93 (0.84 to 1.02)	0.92 (0.83 to 1.02)
Tertile 3	56	11.2 (9.9 to 12.7)	1.06 (0.96 to 1.17)	1.02 (0.92 to 1.12)	1.01 (0.91 to 1.12)
			P_{trend}[c] = .32	P_{trend}[c] = .82	P_{trend}[c] = .51
Lifetime use[d]					
Tertile 1	56	10.2 (8.8 to 11.9)	1.02 (0.93 to 1.12)	0.98 (0.89 to 1.08)	1.00 (Referent)
Tertile 2	56	12.3 (11.1 to 13.7)	1.07 (0.97 to 1.18)	1.02 (0.92 to 1.12)	1.05 (0.94 to 1.16)
Tertile 3	55	10.6 (9.3 to 12.1)	0.99 (0.90 to 1.09)	0.95 (0.86 to 1.05)	0.97 (0.88 to 1.08)
			P_{trend}[c] = .98	P_{trend}[c] = .41	P_{trend}[c] = .47
8-isoprostane (μg/L)					
Nonfarming controls	100	0.53 (0.46 to 0.62)	1.00 (Referent)	—	—
Farming controls	100	0.55 (0.47 to 0.64)	—	1.00 (Referent)	—
Last 12-month use[b]					
Tertile 1	56	0.54 (0.44 to 0.65)	0.99 (0.87 to 1.12)	0.98 (0.85 to 1.14)	1.00 (Referent)
Tertile 2	56	0.60 (0.51 to 0.70)	0.96 (0.84 to 1.10)	0.96 (0.83 to 1.11)	0.98 (0.85 to 1.13)
Tertile 3	56	0.59 (0.49 to 0.70)	1.05 (0.92 to 1.20)	1.04 (0.90 to 1.21)	1.06 (0.92 to 1.23)
			P_{trend}[c] = .43	P_{trend}[c] = .51	P_{trend}[c] = .29
Lifetime use[d]					
Tertile 1	56	0.53 (0.45 to 0.64)	1.02 (0.89 to 1.16)	1.01 (0.87 to 1.17)	1.00 (Referent)
Tertile 2	56	0.61 (0.52 to 0.72)	0.95 (0.84 to 1.09)	0.95 (0.82 to 1.11)	0.94 (0.81 to 1.09)
Tertile 3	55	0.57 (0.48 to 0.69)	1.02 (0.89 to 1.17)	1.02 (0.88 to 1.18)	1.00 (0.86 to 1.16)
			P_{trend}[c] = .90	P_{trend}[c] = .94	P_{trend}[c] = .84
MDA (μM)					
Nonfarming controls	100	1.57 (1.38 to 1.78)	1.00 (Referent)	—	—
Farming controls	100	1.66 (1.47 to 1.88)	—	1.00 (Referent)	—
Last 12-month use[b]					
Tertile 1	56	1.65 (1.44 to 1.88)	1.03 (0.90 to 1.17)	1.00 (0.87 to 1.14)	1.00 (Referent)
Tertile 2	56	1.67 (1.44 to 1.95)	0.97 (0.85 to 1.11)	0.94 (0.82 to 1.08)	0.94 (0.81 to 1.08)
Tertile 3	56	1.75 (1.52 to 2.01)	1.08 (0.95 to 1.24)	1.05 (0.91 to 1.20)	1.05 (0.91 to 1.21)
			P_{trend}[c] = .27	P_{trend}[c] = .47	P_{trend}[c] = .30
Lifetime use[d]					
Tertile 1	56	1.62 (1.38 to 1.91)	1.06 (0.93 to 1.21)	1.02 (0.89 to 1.17)	1.00 (Referent)
Tertile 2	56	1.72 (1.51 to 1.96)	0.98 (0.86 to 1.12)	0.95 (0.83 to 1.09)	0.93 (0.80 to 1.07)
Tertile 3	55	1.74 (1.52 to 1.98)	1.05 (0.92 to 1.20)	1.02 (0.89 to 1.17)	1.01 (0.87 to 1.17)
			P_{trend}[c] = .63	P_{trend}[c] = .93	P_{trend}[c] = .70

[a] Adjusted for age (continuous; years) and natural log-transformed urinary creatinine concentration (continuous; mg/dL). 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; BEEA = Biomarkers of Exposure and Effect in Agriculture; CI = confidence interval; GMR = geometric mean ratio; MDA = malondialdehyde.

[b] Intensity-weighted days of occupational glyphosate use in the last 12 months among glyphosate-exposed farmers (tertile 1 = 0-512; tertile 2 = >512-1320; tertile 3 = >1320-11375), calculated by multiplying the number of days of use in the last 12 months by an exposure intensity score that accounts for factors known to influence pesticide exposure.

[c] Calculated by modeling within-tertile median values as continuous variables.

[d] Intensity-weighted lifetime days of occupational glyphosate use among glyphosate-exposed farmers (tertile 1 = 1321-11440; tertile 2 = >11440-23071; tertile 3 = >23071-244237), calculated by multiplying the total number of lifetime days of use by an exposure intensity score that accounts for factors known to influence pesticide exposure. One participant had missing data on lifetime glyphosate use and was excluded from the analysis.

Supplementary Table 7. Associations of occupational glyphosate use in the last 12 months and cumulative lifetime occupational glyphosate use with urinary oxidative stress biomarker concentrations among recently exposed and high lifetime-exposed farmers in the BEEA study

Glyphosate use	No.	Geometric mean concentration (95% CI)	Fully adjusted GMR (95% CI)[a]		
			Compared with nonfarming controls	Compared with farming controls	Glyphosate-exposed farmers only
8-OHdG (μg/L)					
Nonfarming controls	100	10.2 (9.2 to 11.3)	1.00 (Referent)	—	—
Farming controls	100	10.9 (9.8 to 12.2)	—	1.00 (Referent)	—
Recently exposed[b]					
Last 12-month use[c]					
Tertile 1	33	9.6 (7.7 to 11.9)	0.98 (0.82 to 1.17)	0.97 (0.82 to 1.13)	1.00 (Referent)
Tertile 2	34	11.4 (9.7 to 13.2)	0.96 (0.81 to 1.14)	0.95 (0.81 to 1.10)	0.98 (0.82 to 1.15)
Tertile 3	31	11.3 (9.8 to 13.1)	1.00 (0.83 to 1.19)	0.99 (0.84 to 1.17)	1.04 (0.88 to 1.22)
			P_{trend}[d] = .88	P_{trend}[d] = .94	P_{trend}[d] = .55
Lifetime use[e]					
Tertile 1	33	10.2 (8.4 to 12.4)	0.93 (0.77 to 1.12)	0.93 (0.79 to 1.08)	1.00 (Referent)
Tertile 2	32	11.4 (9.4 to 13.8)	1.03 (0.87 to 1.22)	1.02 (0.88 to 1.19)	1.12 (0.95 to 1.32)
Tertile 3	32	10.4 (9.1 to 11.9)	0.93 (0.77 to 1.11)	0.93 (0.79 to 1.09)	1.02 (0.86 to 1.22)
			P_{trend}[d] = .60	P_{trend}[d] = .61	P_{trend}[d] = .93
High lifetime-exposed[f]					
Last 12-month use[c]					
Tertile 1	24	11.3 (9.2 to 13.9)	1.02 (0.86 to 1.20)	1.02 (0.87 to 1.19)	1.00 (Referent)
Tertile 2	23	12.6 (10.5 to 15.0)	1.13 (0.93 to 1.39)	1.14 (0.95 to 1.36)	1.07 (0.89 to 1.28)
Tertile 3	23	10.9 (8.7 to 13.7)	1.13 (0.91 to 1.40)	1.15 (0.96 to 1.38)	1.03 (0.85 to 1.24)
			P_{trend}[d] = .27	P_{trend}[d] = .14	P_{trend}[d] = .92
Lifetime use[e]					
Tertile 1	24	11.8 (9.8 to 14.3)	1.06 (0.88 to 1.28)	1.09 (0.92 to 1.30)	1.00 (Referent)
Tertile 2	23	12.4 (10.4 to 14.7)	1.08 (0.90 to 1.30)	1.10 (0.93 to 1.30)	0.96 (0.80 to 1.15)
Tertile 3	23	10.6 (8.3 to 13.5)	1.03 (0.84 to 1.25)	1.04 (0.87 to 1.25)	0.91 (0.75 to 1.11)
			P_{trend}[d] = .85	P_{trend}[d] = .57	P_{trend}[d] = .36
8-isoprostane (μg/L)					
Nonfarming controls	100	0.53 (0.46 to 0.62)	1.00 (Referent)	—	—
Farming controls	100	0.55 (0.47 to 0.64)	—	1.00 (Referent)	—
Recently exposed[b]					
Last 12-month use[c]					
Tertile 1	33	0.48 (0.38 to 0.62)	1.25 (1.01 to 1.55)	1.16 (0.93 to 1.46)	1.00 (Referent)
Tertile 2	34	0.63 (0.53 to 0.74)	1.19 (0.97 to 1.46)	1.12 (0.90 to 1.40)	0.96 (0.79 to 1.15)
Tertile 3	31	0.61 (0.48 to 0.76)	1.26 (1.02 to 1.56)	1.17 (0.93 to 1.47)	1.00 (0.83 to 1.20)
			P_{trend}[d] = .19	P_{trend}[d] = .35	P_{trend}[d] = .88
Lifetime use[e]					
Tertile 1	33	0.57 (0.45 to 0.72)	1.31 (1.05 to 1.63)	1.23 (0.97 to 1.54)	1.00 (Referent)
Tertile 2	32	0.55 (0.44 to 0.69)	1.17 (0.96 to 1.42)	1.08 (0.87 to 1.34)	0.84 (0.70 to 1.01)
Tertile 3	32	0.58 (0.47 to 0.72)	1.27 (1.03 to 1.57)	1.17 (0.93 to 1.48)	0.98 (0.80 to 1.18)
			P_{trend}[d] = .21	P_{trend}[d] = .40	P_{trend}[d] = .85
High lifetime-exposed[f]					
Last 12-month use[c]					
Tertile 1	24	0.56 (0.41 to 0.77)	1.02 (0.81 to 1.28)	1.03 (0.80 to 1.33)	1.00 (Referent)
Tertile 2	23	0.65 (0.48 to 0.87)	1.16 (0.88 to 1.52)	1.22 (0.91 to 1.63)	0.98 (0.76 to 1.27)
Tertile 3	23	0.54 (0.40 to 0.74)	1.15 (0.86 to 1.54)	1.18 (0.87 to 1.59)	1.00 (0.76 to 1.30)
			P_{trend}[d] = .36	P_{trend}[d] = .29	P_{trend}[d] = .996
Lifetime use[e]					
Tertile 1	24	0.54 (0.40 to 0.73)	1.02 (0.79 to 1.32)	1.06 (0.80 to 1.40)	1.00 (Referent)
Tertile 2	23	0.64 (0.49 to 0.82)	1.07 (0.83 to 1.36)	1.15 (0.88 to 1.51)	0.96 (0.75 to 1.23)
Tertile 3	23	0.57 (0.39 to 0.82)	1.14 (0.87 to 1.49)	1.15 (0.85 to 1.54)	1.13 (0.86 to 1.49)
			P_{trend}[d] = .30	P_{trend}[d] = .30	P_{trend}[d] = .33

MDA (µM)					
Nonfarming controls	100	1.57 (1.38 to 1.78)	1.00 (Referent)	—	—
Farming controls	100	1.66 (1.47 to 1.88)	—	1.00 (Referent)	—
Recently exposed[b]					
Last 12-month use[c]					
Tertile 1	33	1.53 (1.21 to 1.94)	1.01 (0.79 to 1.30)	1.04 (0.83 to 1.29)	1.00 (Referent)
Tertile 2	34	1.73 (1.46 to 2.05)	0.90 (0.71 to 1.14)	0.97 (0.79 to 1.20)	0.92 (0.74 to 1.16)
Tertile 3	31	1.83 (1.55 to 2.17)	1.08 (0.84 to 1.38)	1.09 (0.87 to 1.37)	1.06 (0.85 to 1.32)
			P_{trend}^{d} = .41	P_{trend}^{d} = .45	P_{trend}^{d} = .40
Lifetime use[e]					
Tertile 1	33	1.69 (1.39 to 2.05)	0.97 (0.75 to 1.25)	1.03 (0.82 to 1.29)	1.00 (Referent)
Tertile 2	32	1.66 (1.32 to 2.09)	0.97 (0.77 to 1.22)	1.01 (0.82 to 1.25)	0.98 (0.79 to 1.23)
Tertile 3	32	1.74 (1.48 to 2.05)	1.01 (0.79 to 1.30)	1.04 (0.83 to 1.31)	1.03 (0.81 to 1.31)
			P_{trend}^{d} = .80	P_{trend}^{d} = .77	P_{trend}^{d} = .75
High lifetime-exposed[f]					
Last 12-month use[c]					
Tertile 1	24	1.76 (1.45 to 2.13)	1.06 (0.83 to 1.35)	1.05 (0.83 to 1.31)	1.00 (Referent)
Tertile 2	23	1.68 (1.38 to 2.05)	1.06 (0.79 to 1.41)	1.05 (0.81 to 1.35)	0.96 (0.74 to 1.24)
Tertile 3	23	1.63 (1.24 to 2.14)	1.08 (0.80 to 1.47)	1.13 (0.87 to 1.46)	1.02 (0.78 to 1.34)
			P_{trend}^{d} = .75	P_{trend}^{d} = .41	P_{trend}^{d} = .77
Lifetime use[e]					
Tertile 1	24	1.56 (1.26 to 1.93)	1.01 (0.77 to 1.32)	1.00 (0.79 to 1.28)	1.00 (Referent)
Tertile 2	23	1.85 (1.50 to 2.29)	1.11 (0.86 to 1.44)	1.12 (0.88 to 1.42)	1.06 (0.82 to 1.37)
Tertile 3	23	1.67 (1.31 to 2.12)	1.05 (0.79 to 1.39)	1.07 (0.83 to 1.38)	1.12 (0.84 to 1.48)
			P_{trend}^{d} = .64	P_{trend}^{d} = .48	P_{trend}^{d} = .44

[a] Adjusted for age (continuous; years), natural log-transformed urinary creatinine concentration (continuous; mg/dL), state (Iowa, North Carolina), season of urine collection (April-September, October-March), time of urine collection (before 4:00 AM, 4:00-5:59 AM, 6:00 AM or later), body mass index (continuous; kg/m^2), smoking status (never, former, current), alcohol consumption (0, 1-6, ≥7 servings in the last 7 days), nonsteroidal anti-inflammatory drug use in the last 7 days (no, yes), infection in the last 7 days (no, yes), history of diabetes (no, yes), history of hypertension and/or heart disease (no, yes), and occupational 2,4-dichlorophenoxyacetic acid use (did not use in the last 12 months, 8-365 days ago, ≤7 days ago). 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; BEEA = Biomarkers of Exposure and Effect in Agriculture; CI = confidence interval; GMR = geometric mean ratio; MDA = malondialdehyde.

[b] Farmers with occupational glyphosate use in the last 7 days.

[c] Intensity-weighted days of occupational glyphosate use in the last 12 months (recently exposed group: tertile 1 = 88-528; tertile 2 = >528-1080; tertile 3 = >1080-11375; high lifetime-exposed group: tertile 1 = 0-440; tertile 2 = >440-1485; tertile 3 = >1485-4800), calculated by multiplying the number of days of use in the last 12 months by an exposure intensity score that accounts for factors known to influence pesticide exposure.

[d] Calculated by modeling within-tertile median values as continuous variables.

[e] Intensity-weighted lifetime days of occupational glyphosate use (recently exposed group: tertile 1 = 1321-6948; tertile 2: >6948-16084; tertile 3: >16084-244237; high lifetime-exposed group: tertile 1 = 11334-17019; tertile 2 = >17019-29987; tertile 3 = >29987-76985), calculated by multiplying the total number of lifetime days of use by an exposure intensity score. One participant in the recently exposed group had missing data on lifetime glyphosate use and was excluded from the analysis.

[f] Farmers with high cumulative lifetime days and intensity-weighted lifetime days of occupational glyphosate use (>80th percentile for both) but no use in the last 7 days.

Supplementary Table 8. Sensitivity analyses for the associations between urinary glyphosate and oxidative stress biomarker concentrations in the BEEA study

		Fully adjusted GMR (95% CI)[b]		
Urinary glyphosate concentration (μg/L)[a]	No.	8-OHdG	8-isoprostane	MDA
White participants only				
Quartile 1 (<LOD-0.289)	91	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	91	1.05 (0.95 to 1.15)	1.02 (0.89 to 1.17)	1.12 (0.98 to 1.27)
Quartile 3 (0.507-0.933)	89	1.14 (1.03 to 1.26)	1.09 (0.94 to 1.26)	1.20 (1.04 to 1.38)
Quartile 4 (0.934-35.2)	90	1.16 (1.04 to 1.30)	1.04 (0.89 to 1.22)	1.22 (1.05 to 1.43)
P_{trend}[c]		.01	.72	.03
Continuous[d]	361	1.03 (1.00 to 1.06)	1.00 (0.96 to 1.05)	1.04 (1.00 to 1.08)
Iowa participants only				
Quartile 1 (<LOD-0.289)	69	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	70	1.05 (0.93 to 1.17)	1.05 (0.90 to 1.22)	1.14 (0.97 to 1.33)
Quartile 3 (0.507-0.933)	68	1.10 (0.98 to 1.24)	1.20 (1.02 to 1.40)	1.19 (1.00 to 1.40)
Quartile 4 (0.934-35.2)	71	1.20 (1.05 to 1.36)	1.12 (0.95 to 1.32)	1.22 (1.03 to 1.46)
P_{trend}[c]		.005	.30	.08
Continuous[d]	278	1.04 (1.00 to 1.08)	1.02 (0.97 to 1.07)	1.03 (0.98 to 1.08)
Enrolled in April-September (farming season)				
Quartile 1 (<LOD-0.289)	57	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	63	0.96 (0.85 to 1.08)	1.03 (0.88 to 1.21)	1.19 (1.00 to 1.41)
Quartile 3 (0.507-0.933)	62	1.16 (1.03 to 1.31)	1.13 (0.96 to 1.33)	1.23 (1.03 to 1.47)
Quartile 4 (0.934-35.2)	73	1.12 (0.99 to 1.27)	1.01 (0.85 to 1.20)	1.31 (1.09 to 1.57)
P_{trend}[c]		.03	.74	.03
Continuous[d]	255	1.03 (0.99 to 1.06)	0.98 (0.94 to 1.03)	1.03 (0.99 to 1.08)
No occupational 2,4-D use in the last 7 days				
Quartile 1 (<LOD-0.289)	90	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	84	1.04 (0.95 to 1.15)	1.00 (0.86 to 1.15)	1.12 (0.97 to 1.29)
Quartile 3 (0.507-0.933)	82	1.13 (1.02 to 1.26)	1.07 (0.92 to 1.25)	1.17 (1.01 to 1.36)
Quartile 4 (0.934-35.2)	82	1.15 (1.03 to 1.29)	1.02 (0.86 to 1.20)	1.21 (1.03 to 1.42)
P_{trend}[c]		.02	.88	.06
Continuous[d]	338	1.03 (1.00 to 1.06)	1.00 (0.95 to 1.04)	1.04 (0.99 to 1.08)
Urinary creatinine within 30-300 mg/dL				
Quartile 1 (<LOD-0.289)	87	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	90	1.04 (0.95 to 1.14)	1.00 (0.88 to 1.14)	1.13 (0.99 to 1.29)
Quartile 3 (0.507-0.933)	89	1.16 (1.04 to 1.28)	1.09 (0.95 to 1.26)	1.22 (1.06 to 1.40)
Quartile 4 (0.934-35.2)	91	1.15 (1.03 to 1.28)	1.05 (0.90 to 1.22)	1.25 (1.07 to 1.45)
P_{trend}[c]		.02	.60	.02
Continuous[d]	357	1.03 (1.00 to 1.06)	1.01 (0.97 to 1.05)	1.04 (1.00 to 1.08)
Excluding outlying oxidative stress biomarker values[e]				
Quartile 1 (<LOD-0.289)	93	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	89	1.04 (0.95 to 1.14)	1.00 (0.88 to 1.13)	1.09 (0.97 to 1.23)
Quartile 3 (0.507-0.933)	91	1.16 (1.05 to 1.28)	1.09 (0.96 to 1.25)	1.19 (1.05 to 1.36)
Quartile 4 (0.934-35.2)	89	1.15 (1.03 to 1.27)	1.06 (0.92 to 1.22)	1.24 (1.08 to 1.42)
P_{trend}[c]		.02	.41	.006
Continuous[d]	362	1.03 (1.00 to 1.06)	1.01 (0.98 to 1.05)	1.05 (1.01 to 1.08)
Excluding controls with home/garden glyphosate use[f]				
Quartile 1 (<LOD-0.289)	64	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	64	1.04 (0.93 to 1.17)	0.95 (0.82 to 1.11)	1.10 (0.95 to 1.28)
Quartile 3 (0.507-0.933)	70	1.13 (1.01 to 1.27)	1.07 (0.92 to 1.24)	1.14 (0.98 to 1.33)
Quartile 4 (0.934-35.2)	84	1.16 (1.02 to 1.31)	1.01 (0.86 to 1.19)	1.23 (1.04 to 1.44)
P_{trend}[c]		.02	.76	.02
Continuous[d]	282	1.02 (0.99 to 1.06)	1.00 (0.96 to 1.04)	1.03 (0.99 to 1.07)

Excluding all participants with home/garden glyphosate use[f]				
Quartile 1 (<LOD-0.289)	55	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Quartile 2 (0.290-0.506)	49	1.11 (0.98 to 1.27)	0.97 (0.81 to 1.15)	1.09 (0.92 to 1.30)
Quartile 3 (0.507-0.933)	54	1.24 (1.08 to 1.41)	1.07 (0.89 to 1.28)	1.18 (0.98 to 1.41)
Quartile 4 (0.934-35.2)	59	1.21 (1.05 to 1.39)	1.03 (0.85 to 1.25)	1.31 (1.08 to 1.58)
P_{trend}[c]		.04	.67	.006
Continuous[d]	217	1.03 (0.99 to 1.07)	1.00 (0.95 to 1.05)	1.05 (1.00 to 1.10)

[a] Quartiles are based on the distribution of urinary glyphosate concentrations among all participants (n = 368) for comparison with results presented in Table 2. 2,4-D = 2,4-dichlorophenoxyacetic acid; 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; BEEA = Biomarkers of Exposure and Effect in Agriculture; CI = confidence interval; GMR = geometric mean ratio; LOD = limit of detection; MDA = malondialdehyde.

[b] Adjusted for age (continuous; years), natural log-transformed urinary creatinine concentration (continuous; mg/dL), state (Iowa, North Carolina), season of urine collection (April-September, October-March), time of urine collection (before 4:00 AM, 4:00-5:59 AM, 6:00 AM or later), body mass index (continuous; kg/m^2), smoking status (never, former, current), alcohol consumption (0, 1-6, ≥7 servings in the last 7 days), nonsteroidal anti-inflammatory drug use in the last 7 days (no, yes), infection in the last 7 days (no, yes), history of diabetes (no, yes), history of hypertension and/or heart disease (no, yes), and occupational 2,4-D use (did not use in the last 12 months, 8-365 days ago, ≤7 days ago).

[c] Calculated by modeling within-quartile median values as a continuous variable.

[d] Per 1-unit increase in $\log_2$-transformed urinary glyphosate concentration, corresponding to a doubling in urinary glyphosate concentration.

[e] Outliers defined as more than 3 standard deviations above the mean (>31.2 µg/L for 8-OHdG, >3.8 µg/L for 8-isoprostane, and >5.8 µM for MDA). The numbers of participants across quartiles are shown for the 8-OHdG analysis (after excluding outlying 8-OHdG values); participant numbers (for quartiles 1, 2, 3, and 4, respectively) after excluding outlying values are 93, 90, 91, and 92 for 8-isoprostane (total n = 366); and 92, 88, 88, and 90 for MDA (total n = 358).

[f] Use in the last 12 months.